ANN MED

informa
healthcare

Annals of Medicine, 2014; Early Online: 1–6

ISSN 0785-3890 print/ISSN 1365-2060 online
DOI: 10.3109/07853890.2014.969303

ORIGINAL ARTICLE

The effects of canrenone on inflammatory markers in patients with metabolic syndrome

Giuseppe Derosa[1,2], Davide Romano[1], Lucio Bianchi[1], Angela D'Angelo[1] & Pamela Maffioli[1,3]

[1]*Department of Internal Medicine and Therapeutics, University of Pavia, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy, and* [2]*Center for the Study of Endocrine-Metabolic Pathophysiology and Clinical Research, University of Pavia, Pavia, Italy, and* [3]*PhD in Experimental Medicine, University of Pavia, Pavia, Italy*

Aim. **To evaluate the effects of canrenone compared to placebo on blood pressure control, some non-conventional biomarkers in cardiovascular stratification, and on metalloproteinases in patients affected by metabolic syndrome.**

Methods. **A total of 156 Caucasian patients were treated with placebo or canrenone, 50 mg once a day, for 3 months and then 50 mg twice a day, till the end of the study.**

We evaluated: systolic (SBP) and diastolic blood pressure (DBP), body weight, body mass index (BMI), fasting plasma glucose (FPG), lipid profile, plasma aldosterone, creatinine, potassium, brain natriuretic peptide (BNP), metalloproteinases 2 and 9 (MMP-2 and -9), lipoprotein (a) (Lp(a)), and serum myeloperoxidase (MPO).

Results. **We observed a significant decrease of SBP and DBP in the canrenone group compared to baseline. Canrenone gave a significant decrease of MMP-2 and -9, Lp(a), and MPO compared to baseline, not observed with placebo. Plasma aldosterone, but not BNP, decreased with canrenone, both compared to baseline and to placebo.**

Conclusion. **Canrenone seems to be effective in reducing blood pressure in patients with metabolic syndrome. Moreover, canrenone seems also to improve MPO, Lp(a), and metalloproteinases in these patients.**

Key words: Canrenone, inflammatory markers, metalloproteinases

Key messages

- Canrenone decreased blood pressure.
- Canrenone gave a significant decrease of metalloproteinase-2 and -9.
- Canrenone decreased lipoprotein(a) and myeloperoxidase.

Introduction

Several studies have demonstrated that physical exercise reduces blood pressure levels in hypertensive subjects and improves control of several well-known risk factors for atherosclerosis such as diabetes mellitus, blood lipid profile, and obesity (1). Due to reduced physical activity, the incidence of metabolic syndrome is high and increasing. Metabolic syndrome refers to a constellation of disturbances including glucose intolerance, central obesity, dyslipidemia (hypertriglyceridemia, elevated non-esterified fatty acids (NEFAs), and decreased high-density lipoprotein (HDL) cholesterol), and hypertension (2). It is well established that it increases the risk for the development of cardiovascular disease, type 2 diabetes, and cancer (3,4). Recent studies have been confirming the positive association between obesity indices and inflammatory markers, mainly C-reactive protein, in women (5), but also other inflammatory markers, both in women and men (6,7). In a previous study we conducted, we showed that lipoprotein (a) (Lp(a)), soluble advanced glycation end-products (sRAGE), soluble CD40 ligand (sCD40L), and serum myeloperoxidase (MPO) are associated with hypertension and may represent an increased risk for cardiovascular diseases (8). Also arterial stiffness seems to play a role: in subjects with ischemic stroke and metabolic syndrome, pulse wave velocity was significantly and positively correlated with body mass index, systolic blood pressure, hypertension, diabetes, glucose blood levels, low density lipoprotein (LDL) cholesterol levels, total cholesterol levels, microalbuminuria, carotid plaque, and previous brain infarct at neuro-imaging (9). Mineralocorticoid levels and activation of mineralocorticoid receptors are increased when metabolic syndrome is diagnosed (10), and this might be in part responsible for the increased cardiovascular risk in these patients (11,12). For this reason, mineralocorticoid receptor antagonists can have a positive effect on blood pressure. Two generations of mineralocorticoid receptor antagonists are currently available: the first generation includes spironolactone and canrenone, while eplerenone is a second-generation mineralocorticoid receptor antagonist, less potent, but more selective, with no active metabolites and a shorter half-life

Correspondence: Giuseppe Derosa, MD, PhD, Department of Internal Medicine and Therapeutics, University of Pavia, Fondazione IRCCS Policlinico S. Matteo, Pavia, P.le C. Golgi, 2 - 27100 Pavia, Italy. Fax: + 39-0382 526259. E-mail: giuseppe.derosa@unipv.it

(Received 17 August 2014; accepted 19 September 2014)

(13). In particular canrenone avoids the formation of intermediate products with anti-androgenic and progestational actions, resulting in a decreased incidence of side effects (14).

The aim of this study was to evaluate the effects of canrenone compared to placebo on glycemia, blood pressure, and some inflammatory parameters in patients affected by metabolic syndrome defined according to Adult Treatment Panel III criteria (15).

Patients and methods

Study design

This double-blind, placebo-controlled study was conducted at the Department of Internal Medicine and Therapeutics, University of Pavia, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy.

The study was conducted according to the Declaration of Helsinki and its amendments and the Good Clinical Practice Guidelines, and the study protocol was approved by the local Ethical Committee. Trial registration: ClinicalTrials.gov NCT02064218.

Patients

One hundred and fifty-six Caucasian patients were enrolled. Patients were >18 years old, of either sex (Table I), affected by metabolic syndrome according to Adult Treatment Panel III criteria (15), naïve to any treatment, and in overweight (BMI ≥ 25, and <30 kg/m^2). Exclusion criteria included secondary hypertension or blood pressure levels $\geq 160/100$ mmHg. At the time of enrollment, all patients underwent an oral glucose tolerance test (OGTT) with 75 g of glucose and glycemia determination at time 0 and after 120 minutes to evaluate if there was a condition of impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or type 2 diabetes mellitus. Patients with type 2 diabetes mellitus diagnosis were excluded. We also excluded patients with a genetic condition affecting lipid metabolism (e.g. familial hypercholesterolemia, type III hyperlipidemia, lipoprotein lipase deficiency, etc.), with cholesterolemia >240 mg/dL, and/or with triglyceridemia >400 mg/dL. Patients with a history of microalbuminuria or nephrotic syndrome were excluded; impaired hepatic function (defined as plasma aminotransferase and/or gamma-glutamyltransferase level higher than the upper limit of normal (ULN) for age and sex), impaired renal function (defined as serum creatinine level higher than the ULN for age and sex), or severe anemia (defined as hemoglobin level lower than 8 g/dL) were exclusion criteria. Patients with thyroid diseases, history of alcohol or drug abuse, neoplastic, infectious or inflammatory or autoimmune disease, or poor mental condition were also excluded. Patients with serious cardiovascular disease (CVD) or New York Heart Association class I–IV congestive heart failure or a history of myocardial infarction or stroke or cerebrovascular conditions (ischemic stroke, hemorrhagic stroke, or transient ischemic attack) within 6 months before study enrollment were also excluded. Women who were pregnant or breastfeeding or of child-bearing potential and not taking adequate contraceptive precautions were also excluded.

Suitable subjects, identified from review of case notes and/or computerized clinic registers were contacted personally or by telephone. All patients provided written informed consent.

Treatments

According to the study design, eligible patients were divided into two groups: patients satisfying the Adult Treatment Panel III metabolic syndrome definition including the blood pressure criteria (BP $\geq 130/85$ mmHg), and patients satisfying the metabolic syndrome definition without the blood pressure criteria (control group) (Table I). The first group was treated with canrenone, 50 mg once a day, for three months and then 50 mg twice a day, with forced titration, till the end of the study; the control group, instead, was treated with placebo. Medications were provided by two doctors that did not know blood pressure values. To maintain the double-blind study design, a third doctor that did not know the study, evaluated the blood pressure. Both canrenone and placebo were supplied as identical, opaque, white capsules in coded bottles to ensure the blind status of the study. Medication compliance was assessed by counting the number of pills returned at the time of specified clinic visits. Throughout the study, we instructed patients to take their first dose of new medication on the day after they were given the study medication. At the same time, all unused medication was retrieved for inventory. All medications were provided free of charge.

Diet and exercise

Subjects were encouraged to follow a controlled-energy diet (near 600 kcal daily deficit) based on American Heart Association (AHA) recommendations (16) that included 50% of calories from carbohydrates, 30% from fat (6% saturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/day and 35 g/day of fiber. Patients were not treated with vitamins or mineral preparations during the study.

Table I. General characteristics of the patients at baseline and after 3 and 6 months in both groups, and ATP III criteria of enrollment.

	Placebo group			Canrenone group		
	Baseline	3 months	6 months	Baseline	3 months	6 months
n	77	75	75	79	78	78
Age (years)	57.8 ± 6.1	–	–	58.2 ± 6.5	–	–
Sex (M/F)	38/39	37/38	37/38	40/39	39/39	39/39
Sm. st. (M/F)	7/8	7/8	8/7	10/8	9/8	9/8
Weight (kg)	79.9 ± 3.0	79.5 ± 2.8	78.9 ± 2.4	78.8 ± 3.2	78.5 ± 2.9	78.1 ± 2.7
Height (m)	1.66 ± 0.05	–	–	1.65 ± 0.05	–	–
ATP III criteria						
WC	Yes	–	–	Yes	–	–
SBP/DBP	No	–	–	Yes	–	–
FPG	Yes	–	–	Yes	–	–
HDL-C	No	–	–	No	–	–
Tg	Yes	–	–	Yes	–	–

Data are means ± SD.
DBP = diastolic blood pressure; FPG = fasting plasma glucose; HDL-C = high density lipoprotein-cholesterol; SBP = systolic blood pressure; Sm. st. = smoking status; Tg = triglycerides; WC = waist circumference.

Standard diet advice was given by a dietician and/or specialist doctor. Dieticians and/or specialist doctors periodically provided instruction on dietary intake recording procedures as part of a behavior modification program and then later used the subject's food diaries for counseling. Individuals were also encouraged to increase their physical activity by walking briskly for 20 to 30 minutes, three to five times per week, or by cycling. The recommended changes in physical activity throughout the study were not assessed.

Assessments

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, vital signs, a 12-lead electrocardiogram, measurements of blood pressure (BP), body weight, body mass index (BMI), waist circumference (WC), fasting plasma glucose (FPG), lipid profile, plasma aldosterone, brain natriuretic peptide (BNP), metalloproteinases 2 and 9 (MMP-2 and -9), Lp(a), and MPO. In order to evaluate the tolerability assessments, all adverse events were recorded.

Clinic BP was obtained in sitting position by standard mercury sphygmomanometer, 24 h after drug intake. Three measurements, taken at 2-min intervals after 10 min of sitting, were averaged and used as the clinic BP reference value. Body mass index was calculated as weight in kilograms divided by the square of height in meters; WC was measured midway between the lateral lower rib margin and the iliac crest.

All plasmatic parameters were determined after a 12-h overnight fast. Venous blood samples were taken for all patients between 8 a.m. and 9 a.m. We used plasma obtained by addition of Na_2-ethylenediamine tetra-acetic acid (EDTA), 1 mg/mL, and centrifuged at 3000 *g* for 15 minutes at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at −80°C for no more than 3 months. All measurements were performed in a central laboratory.

Plasma glucose was assayed by glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and interassay coefficients of variations (CsV) of $<2\%$ (17).

Total cholesterol and triglycerides levels were determined using fully enzymatic techniques (18,19) on a clinical chemistry analyzer (HITACHI 737; Hitachi, Tokyo, Japan); intra- and interassay CsV were 1.0 and 2.1 for total cholesterol measurement, and 0.9 and 2.4 for triglycerides measurement, respectively. High-density lipoprotein cholesterol level was measured after precipitation of plasma apoB-containing lipoproteins with phosphotungstic acid (20); intra- and interassay CsV were 1.0 and 1.9, respectively. LDL-C level was calculated by the Friedewald formula (21).

Aldosterone was measured with a radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA, USA); intra- and interassay CsV were 5.3% and 8.4%, respectively (22).

Plasma BNP was measured using the fully automated Access platform (Triage BNP reagents, Access Immunoassay Systems, REF 98200; Beckman Coulter, Inc., Fullerton, CA, USA). The intra- and interassay CsV for BNP were 4.2% and 6.3%, respectively (22).

Metalloproteinase-2 and MMP-9 levels were determined by a two-site enzyme-linked immunosorbent assay (ELISA) method using commercial reagents (Amersham Biosciences, Uppsala, Sweden). The intra- and interassay CsV for measuring MMP-2 levels were 5.4% and 8.3%, respectively (23). The intra- and interassay CsV to evaluate MMP-9 levels were 4.9% and 8.6%, respectively (24).

Lipoprotein(a) was measured by a sandwich ELISA method that is insensitive to the presence of plasminogen, using the commercial kit Macra-Lp(a) (SDI, Newark, DE, USA); the intra- and interassay CsV of this method were 5% and 9%, respectively (25,26).

Myeloperoxidase was assessed using commercially available ELISA kits according to manufacturer's instructions (R & D Systems, Minneapolis, MN, USA). The intra- and interassay CsV were 7.7% and 8.3%, respectively (27).

Safety measurements

Safety monitoring included physical examination, vital sign assessment, weight, electrocardiogram, adverse events, and laboratory tests. Kidney function was evaluated by measurement of creatinine and potassium, and all adverse events were recorded.

Oral glucose tolerance test

All subjects drank a glass of water (200 mL), in which 75 g of glucose had been dissolved over a period of 5 min in the morning, between 8 a.m. and 9 a.m. after a 12-h fast, and after dietary assessment to ensure a carbohydrate intake >150 g/day over the previous 3 days (28). Normal physical activity was allowed over the previous 3 days. No smoking was allowed during the test. Blood samples were collected in EDTA-containing tubes (Becton Dickinson, Meylan Cedex, France) through a venous catheter from an antecubital vein immediately before and at 120 min after the glucose load for the measurement of the considered parameters of the study.

Statistical analysis

A sample size of 70 patients per group was required to provide 90% power to detect a significant between-group difference. All patients randomized with at least one post-randomization measure were analyzed, i.e. intent-to-treat. Continuous variables were evaluated using a two-way repeated measures analysis of variance (ANOVA). Intervention effects were adjusted for the presence of potential confounding variables using analysis of covariance (ANCOVA). ANOVA was also used to assess the significance of variables, within and between groups. The statistical significance of the independent effects of treatments on the other variables was determined using ANCOVA. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 14.0 (SPSS, Inc., Chicago, IL, USA). All inferential statistical tests were conducted at a significance level of 0.05 (two-sided). Variables were presented as means ± standard deviation (SD) (29).

Results

Study sample

A total of 156 patients were enrolled in the study, 79 were randomized to canrenone and 77 to placebo; 153 patients completed the study. Three patients (two males and one female) did not complete the study and the reasons for premature withdrawal were: lost to follow-up (one female), and informed consent withdrawal (2 males). The characteristics of the patients at baseline are shown in Table I: patients in the placebo group satisfied three criteria for metabolic syndrome (WC, FPG, and triglycerides), while the patients in the canrenone group satisfied these three criteria plus the blood pressure one.

Body mass index and WC

No variations of body weight or waist circumference were recorded in either group.

Blood pressure

No variation of blood pressure was recorded in placebo group. We recorded a decrease of systolic and diastolic blood pressure with canrenone both at 3 and 6 months ($P<0.05$, and $P<0.01$,

Table II. Characteristics of the patients at baseline and after 3 and 6 months in the placebo group.

	Baseline	3 months	6 months
n	77	75	75
M/F	38/39	37/38	37/38
Height	1.66 ± 0.05	1.66 ± 0.05	1.66 ± 0.05
Weight	79.9 ± 3.0	79.5 ± 2.8	78.9 ± 2.4
BMI (kg/m^2)	29.0 ± 0.9	28.8 ± 0.8	28.6 ± 0.8
WC (cm)[a]	109.4 ± 6.4	108.9 ± 5.8	108.5 ± 5.7
SBP (mmHg)	129.5 ± 4.9	128.9 ± 4.1	128.2 ± 3.9
DBP (mmHg)	82.1 ± 3.8	81.9 ± 3.7	80.7 ± 3.4
FPG (mg/dL)[a]	115.9 ± 5.8	114.4 ± 5.1	113.8 ± 4.8
TC (mg/dL)	188.6 ± 19.1	187.9 ± 18.5	185.6 ± 17.7
HDL-C (mg/dL)	44.2 ± 7.1	43.7 ± 6.7	43.6 ± 6.7
Tg (mg/dL)[a]	188.5 ± 34.2	185.1 ± 33.9	183.6 ± 32.8
Creatinine (mg/dL)	0.78 ± 0.30	0.76 ± 0.28	0.77 ± 0.29
Potassium (mEq/L)	4.2 ± 0.5	4.1 ± 0.4	4.4 ± 0.6
Plasma aldosterone (pg/dL)	148.2 ± 22.5	145.5 ± 21.9	142.6 ± 21.5
BNP (pg/mL)	49.1 ± 28.3	48.7 ± 27.8	48.1 ± 27.0
MMP-2 (ng/mL)	635.7 ± 311.5	628.5 ± 305.4	625.6 ± 302.7
MMP-9 (ng/mL)	50.5 ± 15.7	48.1 ± 15.2	47.4 ± 14.8
Lp(a) (mg/dL)	14.5 ± 9.0	14.2 ± 8.8	14.4 ± 8.9
MPO (ng/mL)	410.1 ± 151.2	408.8 ± 149.1	407.4 ± 148.4

Data are means ± SD.
BMI = body mass index; BNP = brain natriuretic peptide; DBP = diastolic blood pressure; FPG = fasting plasma glucose; HDL-C = high density lipoprotein-cholesterol; Lp(a) = lipoprotein (a); MMP-2 = metalloproteinase-2; MMP-9 = metalloproteinase-9; MPO = serum myeloperoxidase; SBP = systolic blood pressure; TC = total cholesterol; Tg = triglycerides; WC = waist circumference.
[a]ATP III criteria for metabolic syndrome diagnosis.

respectively). At randomization, and after 3 months, blood pressure values in the canrenone group were higher than those in the control group ($P < 0.01$, and $P < 0.05$, respectively). No differences between the two groups were recorded at the end of the study (Tables II and III).

Fasting plasma glucose

We recorded a FPG decrease after 6 months with canrenone ($P < 0.05$ versus baseline), but not with placebo. Fasting plasma glucose value recorded with canrenone was lower than the one obtained with placebo at the end of the study ($P < 0.05$).

Lipid profile

No variations of lipid profile were observed with the exception of a decrease of triglycerides in the canrenone group after 6 months compared to baseline ($P < 0.05$) and placebo ($P < 0.05$) (Tables II and III).

Aldosterone and BNP

There was a decrease of aldosterone levels after 3 ($P < 0.05$) and 6 months ($P < 0.01$) with canrenone, but not with placebo, compared to baseline. In group to group comparison, aldosterone was higher in the canrenone group than in the control group at baseline, but lower after 6 months of therapy ($P < 0.05$). BNP did not significantly change during the study (Tables II and III).

Inflammatory parameters

After therapy with canrenone, there was a decrease of MMP-2 and MMP-9 after 3 and 6 months ($P < 0.05$, and $P < 0.01$, respectively for both MMP-2 and MMP-9), not recorded with placebo. In group to group comparison, MMP-2 and MMP-9 recorded in aldosterone group were significantly higher compared to placebo, at baseline ($P < 0.01$ versus placebo), at 3 ($P < 0.01$ versus placebo) and at 6 months ($P < 0.05$ versus placebo). Lp(a) recorded in the canrenone group was higher compared to placebo at baseline ($P < 0.01$) and after 3 ($P < 0.05$) and 6 months ($P < 0.05$). MPO recorded in the canrenone group was higher than the one observed in the placebo group at baseline ($P < 0.01$) and after 3 ($P < 0.01$) and 6 months ($P < 0.05$).

Table III. Characteristics of the patients at baseline and after 3 and 6 months in the canrenone group.

	Baseline	3 months	6 months
n	79	78	78
M/F	40/39	39/39	39/39
Height	1.65 ± 0.05	1.65 ± 0.05	1.65 ± 0.05
Weight	78.8 ± 3.2	78.5 ± 2.9	78.1 ± 2.7
BMI (kg/m^2)	28.9 ± 1.0	28.8 ± 0.9	28.7 ± 0.8
WC (cm)[a]	107.8 ± 6.0	107.1 ± 5.6	106.9 ± 5.4
SBP (mmHg)[a]	144.1 ± 6.1[e]	135.1 ± 5.5[b,d]	125.7 ± 3.4[c]
DBP (mmHg)[a]	90.2 ± 4.2[e]	87.7 ± 3.8[b,d]	81.5 ± 3.6[c]
FPG (mg/dL)[a]	117.7 ± 7.4	110.6 ± 6.2	98.2 ± 5.0[b,d]
TC (mg/dL)	186.8 ± 18.7	185.1 ± 18.2	183.6 ± 17.7
HDL-C (mg/dL)	43.8 ± 6.8	44.1 ± 7.2	44.0 ± 7.1
Tg (mg/dL)[a]	192.1 ± 37.2	182.4 ± 32.5	165.5 ± 25.4[b,d]
Creatinine (mg/dL)	0.80 ± 0.32	0.81 ± 0.33	0.80 ± 0.32
Potassium (mEq/L)	4.0 ± 0.3	4.2 ± 0.5	4.4 ± 0.6
Plasma aldosterone (pg/dL)	171.4 ± 28.5[d]	144.2 ± 22.1[b]	113.1 ± 16.5[c,d]
BNP (pg/mL)	48.8 ± 27.7	48.3 ± 27.2	47.9 ± 26.8
MMP-2 (ng/mL)	1328.1 ± 168.2[e]	1128.5 ± 149.6[b,e]	986.5 ± 127.6[c,d]
MMP-9 (ng/mL)	501.4 ± 54.7[e]	414.3 ± 47.3[b,e]	397.3 ± 41.6[c,d]
Lp(a) (mg/dL)	42.3 ± 52.1[e]	36.5 ± 45.3[d]	29.3 ± 36.5[b,d]
MPO (ng/mL)	772.5 ± 251.6[e]	754.7 ± 243.8[e]	736.1 ± 235.6[b,d]

Data are means ± SD.
BMI = body mass index; BNP = brain natriuretic peptide; DBP = diastolic blood pressure; FPG = fasting plasma glucose; HDL-C = high density lipoprotein-cholesterol; Lp(a) = lipoprotein (a); MMP-2 = metalloproteinase-2; MMP-9 = metalloproteinase-9; MPO = serum myeloperoxidase; SBP = systolic blood pressure; TC = total cholesterol; Tg = triglycerides; WC = waist circumference.
[a]ATP III criteria for metabolic syndrome diagnosis.
[b]$P < 0.05$ versus baseline.
[c]$P < 0.01$ versus baseline.
[d]$P < 0.05$ versus placebo group.
[e]$P < 0.01$ versus placebo group.

Canrenone, but not placebo, decreased Lp(a) and MPO after 6 months of treatment ($P < 0.05$ versus baseline).

Safety measurement

No significant changes of creatinine or potassium were recorded.

Discussion

The results observed in our study regarding the effects of canrenone on body weight, glycemia, lipid profile, and aldosterone are in line with what was already reported in a previous study we conducted (30).

The Anti-remodeling Effect of Canrenone in Patients With Mild Chronic Heart Failure Study (AREA-in-CHF) showed that treatment with canrenone, given in addition to optimal therapy in patients with metabolic syndrome and chronic, stabilized heart failure with reduced ejection fraction, protects against deterioration of myocardial mechano-energetic efficiency, improves diastolic dysfunction, and maximizes the decrease in BNP (31). Differently from what was reported in the AREA-in-CHF trial (31), there were not any variations of BNP levels, but this is probably due to the fact that patients enrolled in the AREA-in-CHF trial were affected not only by metabolic syndrome, but they also had a reduced ejection fraction and a history of heart failure; patients enrolled in the current study, instead, had not. Regarding inflammatory markers, we observed that in patients treated with canrenone, baseline values of MMP-2 and -9, LP(a), and MPO were higher than in patients treated with placebo. This can be explained by the fact that patients randomized to placebo group satisfied three criteria for metabolic syndrome (WC, FPG, and Tg), while the patients in the canrenone group satisfied these three criteria plus the blood pressure one. We have already reported that hypertensive patients had higher levels of Lp(a) and MPO, and that these parameters can be considered new emerging biomarkers of cardiovascular risk (8). The same can be said for MMPs: several studies showed that MMP levels were higher in subjects with hypertension (32,33). The reduction of these markers with canrenone can be attributed to blood pressure reduction, as shown in some previous studies where the reduction of blood pressure led to a decrease of inflammatory markers (34,35). However, previous studies also suggested aldosterone antagonists to have an early suppressive effect on several immunoactive and proinflammatory cytokines, as reported by Sønder et al. (36).

Of course the study has some limitations: for example it was not evaluated if the beneficial effects of canrenone were sustained after the cessation of therapy. Moreover, in the study only some surrogate end-points were evaluated; the efficacy of canrenone in treating the metabolic syndrome should have been better established by hard end-point outcomes data such as death, coronary events, and stroke, which could not be investigated given the short duration of the study.

However, after an accurate research in literature, this study is the first to report the effects of canrenone on inflammatory parameters in patients affected by metabolic syndrome.

Conclusion

Canrenone seems to be effective in reducing blood pressure in patients with metabolic syndrome. Moreover, canrenone seems also to improve MPO, Lp(a), and metalloproteinases in these patients.

Statement of human rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study. Trial registration: ClinicalTrials.gov NCT02064218.

Acknowledgements

Professor Giuseppe Derosa and Dr Pamela Maffioli designed the study, researched data, and wrote the manuscript; Dr Angela D'Angelo processed and analyzed collected samples; Dr Davide Romano and Dr Lucio Bianchi researched data.

Registration number: NCT02064218, ClinicalTrials.gov

Declaration of interest: No funding declared. Giuseppe Derosa, Davide Romano, Lucio Bianchi, Angela D'Angelo, and Pamela Maffioli declare that they have no conflict of interest.

References

1. Pinto A, Di Raimondo D, Tuttolomondo A, Fernandez P, Arnao V, Licata G. Twenty-four hour ambulatory blood pressure monitoring to evaluate effects on blood pressure of physical activity in hypertensive patients. Clin J Sport Med. 2006;16:238–43.
2. Caterson ID, Hubbard V, Bray GA, Grunstein R, Hansen BC, Hong Y, et al.; American Heart Association. Prevention Conference VII: Obesity, a worldwide epidemic related to heart disease and stroke: Group III: worldwide comorbidities of obesity. Circulation. 2004;110:e476–83.
3. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365:1415–28.
4. Galassi A, Reynolds K, He J. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. Am J Med. 2006;119:812–19.
5. Bochud M, Marquant F, Marques-Vidal PM, Vollenweider P, Beckmann JS, Mooser V, et al. Association between C-reactive protein and adiposity in women. J Clin Endocrinol Metab. 2009;94:3969–77.
6. Nijhuis J, Rensen SS, Slaats Y, van Dielen FM, Buurman WA, Greve JW. Neutrophil activation in morbid obesity, chronic activation of acute inflammation. Obesity (Silver Spring). 2009;17:2014–18.
7. Mortensen OH, Nielsen AR, Erikstrup C, Plomgaard P, Fischer CP, Krogh-Madsen R, et al. Calprotectin—a novel marker of obesity. PLoS One. 2009;4:e7419.
8. Derosa G, D'Angelo A, Mugellini A, Pesce RM, Fogari E, Maffioli P. Evaluation of emerging biomarkers in cardiovascular risk stratification of hypertensive patients: a 2-year study. Curr Med Res Opin. 2012; 28:1435–45.
9. Tuttolomondo A, Di Raimondo D, Di Sciacca R, Pecoraro R, Arnao V, Buttà C, et al. Arterial stiffness and ischemic stroke in subjects with and without metabolic syndrome. Atherosclerosis. 2012;225:216–19.
10. Grassi G, Quarti-Trevano F, Seravalle G, Dell'Oro R. Cardiovascular risk and adrenergic overdrive in the metabolic syndrome. Nutr Metab Cardiovasc Dis. 2007;17:473–81.
11. Caprio M, Fève B, Claës A, Viengchareun S, Lombès M, Zennaro MC. Pivotal role of the mineralocorticoid receptor in corticosteroid-induced adipogenesis. FASEB J. 2007;21:2185–94.
12. Frey FJ, Odermatt A, Frey BM. Glucocorticoid-mediated mineralocorticoid receptor activation and hypertension. Curr Opin Nephrol Hypertens. 2004;13:451–8.
13. Hawkins UA, Gomez-Sanchez EP, Gomez-Sanchez CM, Gomez-Sanchez CE. The ubiquitous mineralocorticoid receptor: clinical implications. Curr Hypertens Rep. 2012;14:573–80.
14. Mantero F, Lucarelli G. Aldosterone antagonists in hypertension and heart failure. Ann Endocrinol (Paris). 2000;61:52–60.
15. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106:3143–421.
16. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, et al. Summary of American Heart Association Diet and

Lifestyle Recommendations Revision 2006. Arterioscler Thromb Vasc Biol. 2006;26:2186–91.
17. European Diabetes Policy Group. A desktop guide to type 2 diabetes mellitus. Diabet Med. 1999;16:716–30.
18. Klose S, Borner K. Enzymatische Bestimmung des Gesamtcholesterins mit dem Greiner Selective Analyzer (GSA II). J Clin Chem Clin Biochem. 1978;15:121–30.
19. Wahlefeld AW. Methods of enzymatic analysis: triglycerides determination after enzymatic hydrolysis. 2nd English ed. New York: Academic Press, Inc.; 1974. p. 18–31.
20. Havel RJ, Edr HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest. 1955;34:1345–53.
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
22. Boccanelli A, Cacciatore G, Mureddu GF, de Simone G, Clemenza F, De Maria R, et al. Baseline characteristics of patients recruited in the AREA IN-CHF study (Antiremodelling Effect of Aldosterone Receptors Blockade with Canrenone in Mild Chronic Heart Failure). J Cardiovasc Med (Hagerstown). 2007;8:683–91.
23. Fujimoto N, Mouri N, Iwata K, Ohuchi E, Okada Y, Hayakawa T. A one-step sandwich enzyme immunoassay for human matrix metalloproteinase 2 (72-kDa gelatinase/type IV collagenase) using monoclonal antibodies. Clin Chim Acta. 1993;221:91–103.
24. Fujimoto N, Hosokawa N, Iwata K, Shinya T, Okada Y, Hayakawa T. A one-step sandwich enzyme immunoassay for inactive precursor and complexed forms of human matrix metalloproteinase 9 (92 kDa gelatinase/type IV collagenase, gelatinase B) using monoclonal antibodies. Clin Chim Acta. 1994;231:79–88.
25. Scanu AM, Scandian L. Lipoprotein (a): structure, biology and clinical relevance. Adv Intern Med. 1991;36:249–70.
26. Uterman G, Weber W. Protein composition of lipoprotein (a). J Clin Invest. 1987;80:458–65.
27. Morishita K, Kubota N, Asano S, Kaziro Y, Nagata S. Molecular cloning and characterization of cDNA for human myeloperoxidase. J Biol Chem. 1987;262:3844–51.
28. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care. 2011;34:S4–61.
29. Winer BJ. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill; 1971.
30. Derosa G, Bonaventura A, Bianchi L, Romano D, D'Angelo A, Fogari E, et al. Effects of canrenone in patients with metabolic syndrome. Expert Opin Pharmacother. 2013;14:2161–9.
31. de Simone G, Chinali M, Mureddu GF, Cacciatore G, Lucci D, Latini R, et al.; AREA-in-CHF Investigators. Effect of canrenone on left ventricular mechanics in patients with mild systolic heart failure and metabolic syndrome: the AREA-in-CHF study. Nutr Metab Cardiovasc Dis. 2011;21:783–91.
32. Derosa G, D'Angelo A, Ciccarelli L, Piccinni MN, Pricolo F, Salvadeo S, et al. Matrix metalloproteinase-2, -9, and tissue inhibitor of metalloproteinase-1 in patients with hypertension. Endothelium. 2006; 13:227–31.
33. Morillas P, Quiles J, de Andrade H, Castillo J, Tarazón E, Roselló E, et al. Circulating biomarkers of collagen metabolism in arterial hypertension: relevance of target organ damage. J Hypertens. 2013;31:1611–17.
34. Derosa G, Cicero AF, Carbone A, Querci F, Fogari E, D'Angelo A, et al. Different aspects of sartan + calcium antagonist association compared to the single therapy on inflammation and metabolic parameters in hypertensive patients. Inflammation. 2014;37:154–62.
35. Derosa G, Maffioli P, Ferrari I, Palumbo I, Randazzo S, Fogari E, et al. Different actions of losartan and ramipril on adipose tissue activity and vascular remodeling biomarkers in hypertensive patients. Hypertens Res. 2011;34:145–51.
36. Sønder SU, Mikkelsen M, Rieneck K, Hedegaard CJ, Bendtzen K. Effects of spironolactone on human blood mononuclear cells: mineralocorticoid receptor independent effects on gene expression and late apoptosis induction. Br J Pharmacol. 2006;148:46–53.